Story by Kathy Wythe

Beating Bacteria

Scientists work to understand and track bacteria in water

A big production is underway in Texas—not a theatrical production but a scientific one, investigating bacterial pollution in fresh waters.

Bacteria is the No. 1 pollutant of Texas water. Recreation in 274 waterbodies, a majority of which are freshwater streams, rivers, and lakes, is impaired because of bacteria contamination, according to the state's 2008 impaired water list. As a result, much of the ongoing scientific investigation has focused on freshwater recreation.

To determine if fresh water is impaired for contact recreational activities such as swimming and wading, Texas water managers and scientists primarily use *E. coli* bacteria as an indicator of possible fecal contamination. Water with fecal contamination may contain pathogens—not only bacteria but viruses and parasites as well. These pathogens can cause illnesses in swimmers and anyone else who swallows the water, according to U.S. Environmental Protection Agency (EPA) studies.

"The concern is that there are elevated numbers of *E. coli* in many of the streams in Texas," said Dr. Terry Gentry, assistant professor of soil and aquatic microbiology in Texas A&M University's Department of Soil and Crop Sciences, "and this (large number) indicates that there may potentially be pathogens in the water that can cause disease."

Bacteria are so prevalent in the water because they are found in fecal wastes of all warm-blooded animals, said Dr. Larry Hauck, lead scientist of Tarleton State University's Texas Institute for Applied Environmental Research (TIAER). Hauck is involved in several bacterial prevention pollution projects.

"Anything from a diaper thrown out in the parking lot at a mall to birds and livestock at water sources can contribute bacteria to the environment," he said.

This fecal contamination can find its way into the state's streams, rivers, and lakes through runoff from the surrounding land, inadequate treatment of wastewater, and failing septic systems.

Bacteria Task Force Recommendations

Acknowledging the enormity of the bacteria problem within the state, in September 2006, TCEQ and the Texas State Soil and Water Conservation Board (TSSWCB) established a joint Task Force on Bacteria Total Maximum Daily Loads (TMDLs) to make recommendations to strengthen the agencies' efforts in cleaning up bacteria-contaminated waters.

Dr. Allan Jones, formerly of the Texas Water Resources Institute (TWRI) and now of the Texas AgriLife Research and Extension Urban Solutions Center in Dallas, was chair of the task force. Other members were Dr. George Di Giovanni of Texas AgriLife Research Center at El Paso; Hauck of TIAER; Dr. Joanna Mott of Texas A&M University – Corpus Christi; Dr. Hanadi Rifai of the University of Houston; Dr. Raghavan Srinivasan of Texas A&M University's Spatial Science Laboratory; and Dr. George Ward of The University of Texas at Austin. More than 50 other professionals contributed to the task force's report. ⇨

In its 2007 report, the task force made recommendations for implementing bacteria TMDLs and implementation plans (I-Plans) as well as suggestions for research needed to strengthen the scientific tools available for TMDL and I-Plan development.

According to TCEQ, a TMDL is like a budget for pollution. It is a calculation of the maximum amount of a pollutant, such as bacteria, that a water body can receive from all sources and still meet water quality standards. An I-Plan puts the TMDL into action by outlining the steps necessary to reduce pollutant loads through regulatory and voluntary activities.

The task force recommended that the state agencies follow a three-tier approach to implementing bacteria TMDLs. (See box for recommendations and descriptions of tiers on page 16.)

"Basically, the task force recommended simple, less time-consuming, and less costly processes for the first tier, with increasingly complex methods used for more complicated TMDLs," Jones said.

"The three-tiered approach to developing bacteria TMDLs and I-Plans incorporated adaptive management, and phased implementation to the extent allowable by EPA," he said. "The objectives of Tiers 1 and 2 are to ensure that each TMDL is developed using a scientifically credible, cost-effective process with strong stakeholder involvement."

According to Ron Stein, TMDL team leader at TCEQ, his agency is following most of the task force recommendations for its current and future TMDLs and I-Plans. "Based on work we (TCEQ) have done across the state, it is apparent that the best approach for dealing with contact recreation impairments for bacteria is essentially following the Tier 1 recommendations from the task force report," he said. "This means to use the simplest method you can to determine the TMDL."

TCEQ currently has 13 TMDL projects for bacteria that are addressing 64 of the impaired water body segments; all are using the Tier 1 process, he said.

Hauck and his team at TIAER are working with TCEQ to develop TMDLs in several watersheds, including the Upper Trinity River, Carters and Burton creeks in Brazos County, and Cottonwood Branch and Grapevine Creek in Tarrant and Dallas counties.

Projects: applying the science to the streams

As TCEQ and others are following the task force's recommendations for developing TMDLs and I-Plans, other scientists are refining the scientific tools as suggested in the task force report.

One project focusing on the scientific tools as well as the recommendations of the task force is the *Fate and Transport of E. coli in Rural Texas Landscapes and Streams.* This project is an assessment demonstration project funded by TSSWCB through a Clean Water Act §319(h) nonpoint source grant from the EPA and managed by TWRI. Dr. R. Karthikeyan, assistant professor, and Dr. Saqib Mukhtar, associate professor and Texas AgriLife Extension Service specialist in Texas A&M's Department of Biological and Agricultural Engineering; Dr. Roel Lopez, associate director of Texas A&M's Institute of Renewable Natural Resources; Srinivasan, director of Texas A&M's Spatial Sciences Laboratory; and Dr. Daren Harmel, agricultural engineer for USDA's Agricultural Research Service in Temple, are working together on the project.

One component of the project entails the scientists conducting a sanitary survey of a demonstration watershed to identify the specific animals that are contributing *E. coli*. Israel Parker, Lopez's graduate student, is trapping wildlife frequenting the study area. The wildlife fecal samples are then taken to the lab where the *E. coli* is extracted and counted.

"What we are finding is that *E. coli* counts from feces of armadillos, raccoons, and opossums are significantly higher compared to the *E. coli* counts from feces of cattle, and that median *E. coli* concentrations varied with age and gender," Mukhtar said. ⇨

Story continued on page 15.

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sultants conduct one to three surveys on the water body. They determine if there is any recreation activity on the water and/or public access to the water and measure the flow and depth of the water.

The surveyors also look at historical records and interview people who know the area.

"You can only get so much information with surveys," he said. "Observations from local people are important."

Dr. Larry Hauck of Tarleton State University's Texas Institute of Applied Environmental Research and his staff are conducting RUAAs in the Dallas/Fort Worth area and in the Atascosa River watershed.

"The premise is that through site visits, looking at historical records, and talking to local people, you can reconstruct what recreational activities have happened in the past and what is occurring in the present at these various stream systems you are studying," Hauck said. "We are actually gathering data that will indicate what the true level of recreational use occurring, as determined from studies."

water quality standards

Water quality standards are the foundation for managing surface water quality. A standard consists of two parts:

- a use, or the purposes for which surface water will be used
- the criteria or the indicators used to determine if the use is met

Davenport said the two agencies have more than 120 RUAAs being conducted. Depending on the results of the RUAAs and standards revisions, water bodies could be put into one of the four proposed categories of contact recreation, and, depending on the associated bacteria counts, some of the water bodies may no longer be listed on the state's impaired water body list.

The proposed expanded contact recreation use and water quality standards, along with the RUAAs, will provide a better starting point for developing TMDLs, TMDL I-Plans, and WPPs, paving the way for improved water quality in Texas.

e work

"Prior to this study, we did not have any data comparing feces of cattle versus wildlife to determine what kind of *E. coli* loads we had," he said, adding that such findings have not been published previously in the literature.

The scientists are also identifying the different types of land uses throughout the watershed, which helps determine what animals may be on the land. For example, Karthikeyan said, if they know the watershed has cattle ranches, they can estimate the number of cattle on the land and calculate the potential amount of bacteria from the cattle wastes.

Information obtained during the sanitary survey provides input data for the modeling tool SELECT—Spatially Explicit Load Enrichment Calculation Tool. This model was developed and applied by Karthikeyan's graduate students Aarin Teague, Kendra Reibschleager, and Kyna McKee to analyze the land use and animal and human sources in the watershed to determine the potential bacteria sources and their contributions. SELECT then helps the researchers develop a pie chart with the different percentage contributions from each potential source.

"We wanted to see what sources are really contributing, and what percent each source is really contributing to the creek," Karthikeyan said. ➩

In the past, when load or pollutant reductions were calculated for the TMDL, the same amount of reduction was applied to all sources throughout the watershed, Karthikeyan said. Instead of making every contributor reduce its load the same amount, with SELECT and the development of the pie chart, the contributor is given the load reduction based on what it is actually contributing.

These researchers also are interested in how *E. coli* is transported into the water, how long it lives, and how it grows outside in the environment, and the environmental factors that influence these processes. Biological and Agricultural Engineering graduate students Reema Padia and Meghan Gallagher are looking at the different conditions that trigger the growth, survival, and regrowth of *E. coli* bacteria and characterizing optimal growth conditions for different *E. coli* isolates from different sources.

Mukhtar said the growth and survival of *E. coli* from animal feces were tested in soil with 25 percent moisture content (nearly dry or aerobic conditions), 57 percent moisture content, and 83 percent moisture content (nearly saturated or anaerobic conditions). "Our results show that *E. coli* from both cattle and wildlife had greater growth at 25 percent soil moisture content rather than the expected higher moisture environment of 75 percent or more," he said. "This finding verified the facultative behavior (growth and survival under both aerobic and anaerobic conditions) of *E. coli* contributing to accelerated growth levels at a cooler temperature (20 C) and under nearly aerobic conditions."

The scientists are also looking *E. coli* being re-suspended or dispersed again in streams, suspected as a significant source of *E. coli*. Karthikeyan said streams with high flows always spike in *E. coli* concentrations, and the team is trying to determine if the spike is from *E. coli* in runoff or from *E. coli* in the creek sediments that are re-suspended.

"An indicator organism of fecal contamination should not live and reproduce in the environment for an extended period of time after it leaves the gut of an animal," he said. "If *E. coli* does, it may not be a good indicator."

Recommendations of Bacterial TMDL Task Force

The Task Force recommended a three-tier approach to implementing bacteria TMDLs and I-Plans:

Tier 1 is a one-year process that includes the following steps:

1) Form representative stakeholder group

2) Develop comprehensive GIS of the watershed

3) Survey potential bacterial sources

4) Calculate load duration curves from existing monitoring data

5) Analyze data collected by agency personnel and stakeholders

After reviewing information from Tier 1, the group may choose to complete and submit a draft TMDL for agency approval, request an evaluation of the designated use of the water body (a use attainability analysis), or proceed to Tier 2.

Tier 2 is a one- to two-year effort with the following steps:

1) Collect targeted monitoring data to fill gaps in previously collected data

2) Conduct qualitative library-independent BST and limited library-dependent BST analysis to determine whether humans and/or a few major classes of animals are sources

3) Develop simple spatially explicit or mass balance models of bacteria in the watershed

4) Analyze data

After analysis of Tier 1 and Tier 2 data, the group may chose to complete and submit the draft TMDL (or I-Plan if a TMDL was developed after Tier 1), request an evaluation of the designated use (an use attainability analysis), or initiate a "phased TMDL" and proceed with Tier 3 analysis.

When finished, the project's results will help decrease the uncertainties in estimating the *E. coli* load from various sources and help simulate the fate and transport processes of *E. coli* in watersheds and streams, the researchers said.

Other TWRI-managed projects using the task force recommendations include the *Modeling Support* and *Bacterial Source Tracking for Big Cypress Creek*, *Development of a Watershed Protection Plan for Attoyac Bayou*, *Bacteria Assessment and Modeling Support for Buck Creek Watershed Protection Plan Development*, and *Little Brazos River Bacteria Assessment* projects. TSSWCB funds these projects with either federal Clean Water Act §319(h) grants or state general revenue appropriated by the Legislature.

Bacterial Source Tracking

The task force also recommended ongoing research into bacterial source tracking (BST). BST uses genetic fingerprinting techniques similar to forensics or paternity testing to identify bacterial strains that are host-specific, Gentry said.

"The technique generates unique fingerprints of *E. coli* from each potential source," he said, "so a fingerprint of *E. coli* from a cow is going to look different than that from a hog."

The task force recommended BST for the second and third tier of TMDL development and in I-Plans. Library-independent methods were suggested for preliminary qualitative analyses, and the more expensive and time-consuming library-dependent methods were suggested if more definitive data are required for TMDL or I-Plan development.

For several years, task force member Di Giovanni, professor and Faculty Fellow in environmental microbiology, has led the development of BST research in Texas in his laboratory at the Texas AgriLife Research and Extension Center at El Paso. (See related story on page 20.) Di Giovanni and Gentry are expanding the statewide library, developed through Di Giovanni's previous work, incorporating *E. coli* isolates from different animals and different geographical locations.

To develop the Texas Known Source Library or library-dependent BST, Di Giovanni explained, they collect samples from known fecal sources, such as wildlife, pets, domestic animals, livestock, and wastewater samples that are of human origin.

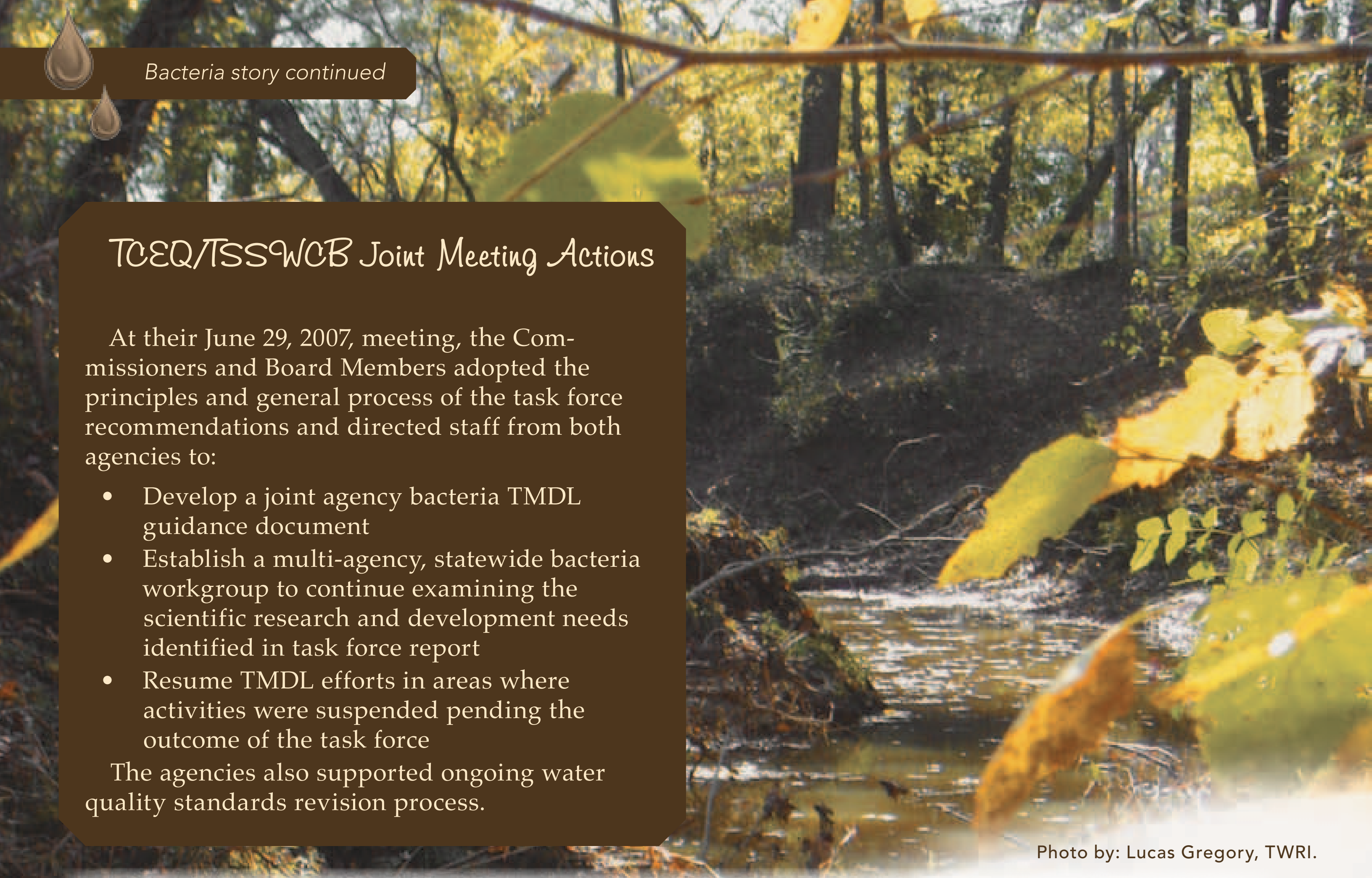
"We then isolate *E. coli* from those samples and type them with our typing or fingerprinting techniques," he said. "And we create a library of *E. coli* from these known source samples. Then we get a water sample and we isolate the *E. coli* that are from unknown origins. We match these fingerprints (of *E. coli*) up with an isolate in our library and that identifies the source."

Library-independent BST uses similar molecular techniques to detect a different group of bacteria, *Bacteroidales*, ⇨

Tier 3 is a two- to three-year process that includes:

1) **Continue strong stakeholder involvement**
2) **Implement more extensive targeted monitoring**
3) **Conduct quantitative library-dependent BST analysis**
4) **Develop a detailed hydrologic/water quality model for the watershed**
5) **Analyze data**

Tier 3 should be implemented only when this level of detailed analysis is needed for I-Plan development or for TMDL development for particularly complex watersheds for which consensus cannot be reached after Tier 2.



TCEQ/TSSWCB Joint Meeting Actions

At their June 29, 2007, meeting, the Commissioners and Board Members adopted the principles and general process of the task force recommendations and directed staff from both agencies to:

- Develop a joint agency bacteria TMDL guidance document
- Establish a multi-agency, statewide bacteria workgroup to continue examining the scientific research and development needs identified in task force report
- Resume TMDL efforts in areas where activities were suspended pending the outcome of the task force

The agencies also supported ongoing water quality standards revision process.

Photo by: Lucas Gregory, TWRI.

from certain animal populations in the water samples. *Bacteroidales* are more abundant in feces than *E. coli* and, because they are less tolerant of oxygen, are less likely to multiply in the environment, Gentry said. The DNA is extracted from water samples and tested for genetic markers that are already developed for *Bacteroidales* associated with humans, ruminants (such as cattle, deer, and sheep), horses, and hogs in addition to new genetic markers for other sources as they are developed.

The disadvantage of using *Bacteroidales*, Di Giovanni said, is currently no genetic markers have been established for most wildlife, which researchers are finding to be significant contributors to the bacteria pollution. "It doesn't encompass all the potential sources we're interested in, and that's the serious weakness of it," he said.

With a grant from TSSWCB, funded with state general revenue appropriations, Gentry equipped a BST lab equivalent to the El Paso lab. Through different projects throughout the state, both labs are collaborating to expand and verify the Texas Known Source Library.

For example, Gentry said, his lab, along with Di Giovanni's, is testing the library with *E. coli* isolates from different geographic regions in Texas to determine if the existing library is sufficiently representative or if additional *E. coli* isolates need to be added to the library.

"As part of several projects in multiple watersheds, we are pulling approximately 100 *E. coli* isolates over time from each watershed and comparing them to the statewide known-source library," Gentry said. "We are also collecting approximately 250 water samples from each of the watersheds and screening those for *Bacteroidales*."

Gentry said he is using the combined approach (library-dependent and library-independent) on several projects, including the *Modeling Support and Bacterial Source Tracking for Big Cypress Creek Bacteria Assessment* and the Attoyac Bayou and Little Brazos River projects.

Complicated waters

With results from these projects showing that wildlife is a major contributor in many rural watersheds, the solutions may be different from expected.

"People often automatically think that high *E. coli* counts in water indicate contamination from humans, grazing livestock, or concentrated animal feeding operations," Gentry said. "While these can be major sources, as more data is coming in, we are seeing a large wildlife contribution (in the watersheds he is investigating). If you determine that a substantial portion of the bacterial contamination is coming from human-associated sources such as malfunctioning septic systems or livestock, you can repair or improve the septic system or implement best management practices to reduce or eliminate the contamination. However, it is less clear what to do about the wildlife contributions to water quality impairments."

Wildlife expert Lopez said there are options to address possible wildlife contamination. "Good range management can deal with any potential contributions from free-ranging wildlife," he said.

For Karthikeyan, the key is verifying the models. Although there "will always be uncertainty and variability" in determining the source of bacterial pollution, by developing more exacting analysis techniques, he said, they can more confidently convey to stakeholders the potential sources.

"Describing fate and transport of *E. coli* in a watershed is really a complex process, but we are doing the best we can," said Karthikeyan, who is working with TSSWCB to use SELECT in over a half dozen watersheds across the state.

"We are providing solutions based on science not just stats and graphs. We are getting the best science possible."

Even without all the answers, Stein of TCEQ said the new way of developing TMDLs and I-Plans with more people involved is "instrumental and vital" to improving water quality around the state.

"There is vast amount of effort across the state to put in place activities that will improve water quality," Stein said. "The state is working quite diligently to get people engaged in improving water quality in their watersheds."

Involvement from local residents is important, Stein said, because "if you can get people in the watershed engaged in thinking how they can improve water quality, you are getting the best plan possible. You are getting the people living in the watershed who know the watershed and know what is going on to develop the plan, and they are best equipped to do that."

For more information about TWRI's bacteria-related projects, visit *http://twri.tamu.edu.* Links to TCEQ's and TSSWCB's water quality information can also be found on the TWRI Web site.

Draft Definitions (2010 TSWQS Revision)

- ***Primary contact recreation:*** Activities presumed to involve a significant risk of ingestion of water (e.g., wading by children, swimming, water skiing, diving, tubing, surfing, and whitewater kayaking, canoeing, and rafting).
- ***Secondary contact recreation 1:*** Activities that commonly occur but have limited body contact incidental to shoreline activity (e.g. fishing and boating). These activities are presumed to pose a less significant risk of water ingestion than primary contact recreation but more than secondary contact recreation 2.
- ***Secondary contact recreation 2:*** Activities with limited body contact incidental to shoreline activity (e.g. fishing and boating) that are presumed to pose a less significant risk of water ingestion than secondary contact recreation 1. These activities occur less frequently than secondary contact recreation 1 due to physical characteristics of the water body or limited public access.
- ***Noncontact recreation:*** Activities that do not involve a significant risk of water ingestion and where primary and secondary contact recreation should not occur because of unsafe conditions, such as ship and barge traffic. Activities would include those with limited body contact incidental to shoreline activity, such as birding, hiking, and biking.

The proposed standards and additional summary information are available at: http://www.tceq.state.tx.us/permitting/water_quality/stakeholders/2010standards.html.